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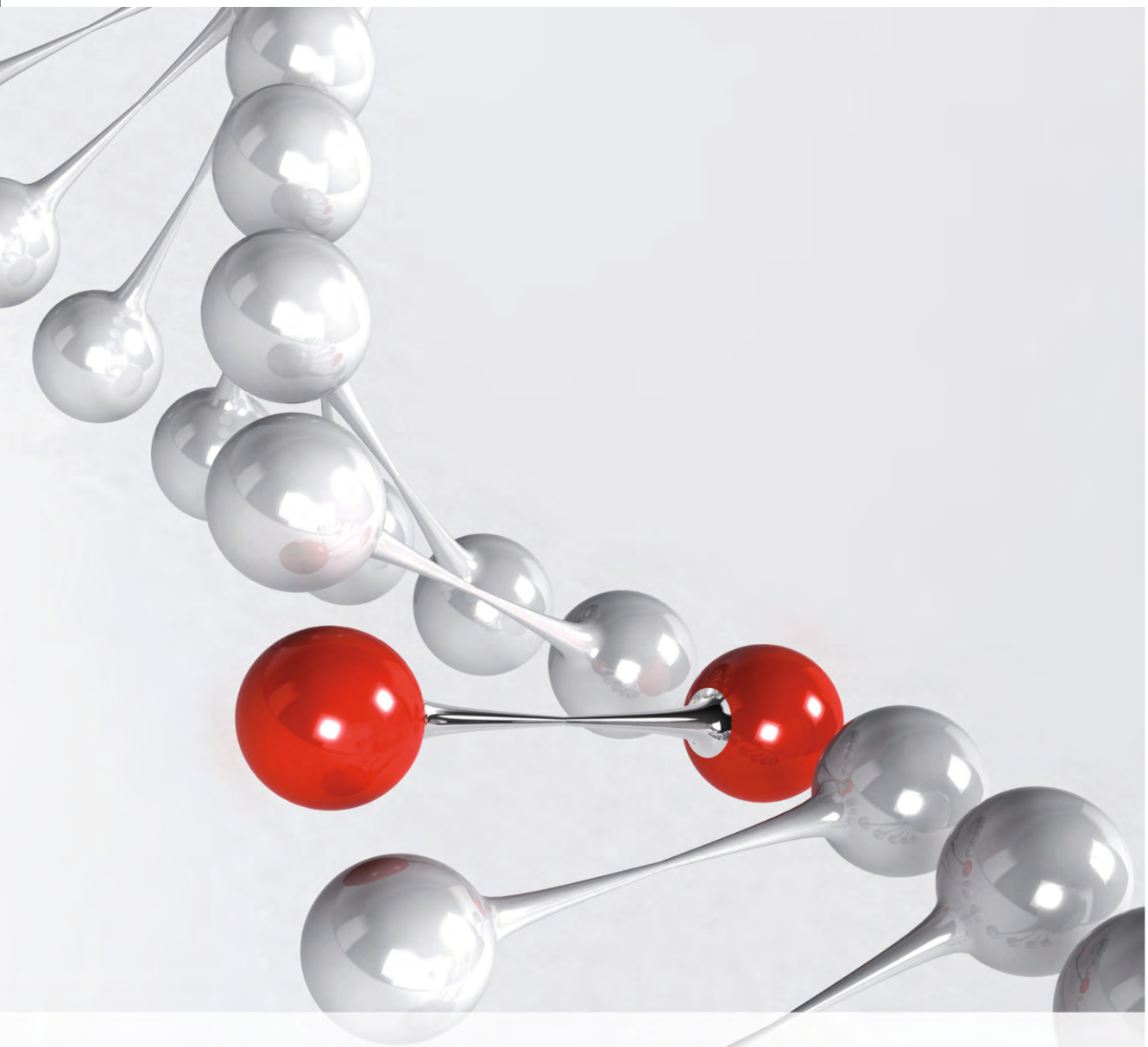

User manual
InviTrap® RNA Tissue HTS 96 Kit/ C

for purification of total RNA from 1-10 mg fresh or frozen tissue sample - without DNase digestion - in a 96-well format using a centrifuge

CE
IVD

REF 7062300x00

STRATEC Molecular GmbH, D-13125 Berlin

Instruction for InviTrap® RNA Tissue HTS 96 Kit/ C

The **InviTrap® RNA Tissue HTS 96 Kit/ C** is designed for isolation of high quality total RNA from 96 samples of fresh or frozen tissue sample.
No enzymatic step (e.g. DNase digestion) to remove contaminating genomic DNA is necessary. Due to the high purity, the isolated total RNA is ready to use for a broad panel of downstream applications.

The **InviTrap® RNA Tissue HTS 96 Kit/ C** is optimized for use on a centrifuge

CE **IVD** Compliance with EU Directive 98/79/EC on *in vitro* medical devices.

Not for in vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized

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The Invisorb® technology is covered by patents and patent applications: US 6,110363, US 6,043,354, US 6,037,465, EP 0880535, WO 9728171, WO 9534569, EP 0765335, DE 19506887, DE 10041825.2, WO 0034463.

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The PCR process is covered by US Patents 4,683,195, and 4,683,202 and foreign equivalents owned by Hoffmann-La Roche AG.

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Kit Content of InviTrap® RNA Tissue HTS 96 Kit/ C

Store all kit components at room temperature.

	2 x 96 RNA preps	4 x 96 RNA preps	24 x 96 RNA preps
Catalogue No.	7062300200	7062300300	7062300400
Lysis Solution DCT	120 ml	220 ml	2 x 650 ml
Binding Buffer R	36 ml (final volume 120 ml)	60 ml (final vol. 1 x 200 ml)	2 x 180 ml (final vol. 2 x 600 ml)
Elution Buffer R	30 ml	60 ml	200 ml
Wash Buffer R1	60 ml (final volume 120 ml)	120 ml (final volume 240 ml)	2 x 350 ml (final vol. 2 x 700 ml)
Wash Buffer R2	30 ml (final vol. 150 ml)	2 x 30 ml (final vol. 2 x 150 ml)	2 x 180 ml (final vol.2 x 900 ml)
2.0 ml Collection Plate	2 x 3	3 x 4	18 x 4
Elution Plate L	2	4	24
RNA Binding Plate A	2	4	6 x 4
Plate Lid	2	4	24
Manual	1	1	1
Initial steps	add 84 ml 96 – 100 % ethanol to the bottle **Binding Buffer R** add 60 ml 96 – 100 % ethanol to the bottle **Wash Buffer R1** add 120 ml 96 – 100 % ethanol to the bottle **Wash Buffer R2**	add 140 ml 96 – 100 % ethanol to each bottle **Binding Buffer R** add 120 ml 96 – 100 % ethanol to each bottle **Wash Buffer R1** add 120 ml 96 – 100 % ethanol to each bottle **Wash Buffer R2**	add 420 ml 96 – 100 % ethanol to each bottle **Binding Buffer R** add 350 ml 96 – 100 % ethanol to each bottle **Wash Buffer R1** add 720 ml 96 – 100 % ethanol to each bottle **Wash Buffer R2**

Symbols

Lot number

Catalogue number

Expiry date

Consult operating instructions

Temperature limitation

Do not reuse

Storage

The InviTrap® RNA Tissue HTS 96 Kit/ C should be stored dry, at room temperature.
It is stable for at least 12 months under these conditions.
Room temperature (RT) is defined as range from 15 - 30°C.

Before every use make sure that all components have room temperature. If there are any precipitates within the provided solutions solve these precipitates by warming carefully.

Quality control and product warranty

STRATEC Molecular warrants the correct function of the **InviTrap® RNA Tissue HTS 96 Kit/ C** for applications as described in this manual. Purchaser must determine the suitability of the Product for its particular use. Should any Product fail to perform the applications as described in the manual, STRATEC Molecular will check the lot and if STRATEC Molecular investigates a problem in the lot, STRATEC Molecular will replace the Product free of charge.
STRATEC Molecular reserves the right to change, alter, or modify any Product to enhance its performance and design at any time.
In accordance with STRATEC Molecular's ISO 9001-2000 and ISO EN 13485 certified Quality Management System the performance of all components of the **InviTrap® RNA Tissue HTS 96 Kit/ C** have been tested separately against predetermined specifications routinely on lot-to-lot to ensure consistent product quality.
If you have any questions or problems regarding any aspects of **InviTrap® RNA Tissue HTS 96 Kit/ C** or other STRATEC Molecular products, please do not hesitate to contact us. A copy of STRATEC Molecular's terms and conditions can be obtained upon request or are presented at the STRATEC Molecular webpage.

For technical support or further information please contact:

from Germany	**+49-(0)30-9489-2901/ 2910**
from abroad	**+49-(0)30-9489-2907**

or contact your local distributor .

Intended use

The **InviTrap® RNA Tissue HTS 96 Kit/ C** has been designed to isolate high yield of cellular total RNA from 1 mg up to 10 mg fresh or frozen tissue sample in a 96-well format.

For reproducible and high yields an appropriate sample storage and quick operation under the rules for RNA operation is essential. The purified RNA can be used for *in vitro* diagnostic analysis.

The **InviTrap® RNA Tissue HTS 96 Kit/ C** is optimized for use with a centrifuge.

THE PRODUCT IS INDENTED FOR USE BY PROFESSIONAL USERS ONLY, SUCH AS TECHNICIANS, PHYSICIANS AND BIOLOGISTS TRAINED IN MOLECULAR BIOLOGICAL TECHNIQUES. It is designed to be used with any downstream application employing enzymatic amplification or other enzymatic modifications of RNA followed by signal detection or amplification. Any diagnostic results generated by using the sample preparation procedure in conjunction with any downstream diagnostic assay should be interpreted with regard to other clinical or laboratory findings.

To minimize irregularities in diagnostic results, adequate controls for downstream applications should be used.

The kit is in compliance with EU Directive 98/79/EC on in vitro medical devices. But it is not for in-vitro diagnostic use in countries where the EU Directive 98/79/EC on in vitro medical devices is not recognized.

Product use limitation

The kit is neither validated for the isolation of total RNA from serum, plasma, bacteria or yeast cells, nor for viruses. The performance of the kit in isolating and purifying total RNA from fecal samples has not been evaluated.

The kit was not tested on its ability to desalinate RNA or for RNA purification from enzymatic reactions, like Proteinase digestion, RNA ligation or labeling reactions.

The included chemicals are for one time use, plates can be used several times, if not all wells were used in first run and unused wells were sealed during the process and no contamination occurred.

When changing the starting material or the flow trace, no guarantee in operability is issued.

The user is responsible to validate the performance of the STRATEC Molecular kits for any particular use, since the performance characteristics of our kits have not been validated for any specific application. STRATEC Molecular kits may be used in clinical diagnostic laboratory systems after the laboratory has validated the complete diagnostic system as required by CLIA' 88 regulations in the U.S. or equivalents in other countries.

All products sold by STRATEC Molecular are subjected to extensive quality control procedures (according to ISO 9001-2000 and ISO EN 13485:2003) and are warranted to perform as described when used correctly. Any problems should be reported immediately.

The chemicals and plastic parts are for laboratory use only, they must be stored in the laboratory and must not use for purposes other than intended.

The kit contents are unfit for consumption.

Safety information

When working with chemicals, always wear a suitable lab coat, disposable gloves, and protective goggles. Avoid skin contact! Heed the legal requirements for working with biological material!

For more information, please consult the appropriate material safety data sheets (MSDS). These are available online in convenient and compact PDF format at **www.stratec.com** for each STRATEC Molecular kit and kit component.

If the buffer bottles are damaged or leaking, wear gloves, and protective goggles when discarding the bottles in order to avoid any injuries.

STRATEC Molecular has not tested the liquid waste generated by the **InviTrap® RNA Tissue HTS 96 Kit/ C** procedures for residual infectious materials. Contamination of the liquid waste with residual infectious materials is highly unlikely, but cannot be exclude completely. Therefore, liquid waste must be considered infectious and be handled and discarded according to local safety regulation.

Below are listed the European Community risk and safety phrases for the components of the **InviTrap® RNA Tissue HTS 96 Kit/ C** to which they apply.

Lysis Solution DC, **Wash Buffer R1** and **Lysis Solution D** contain a chaotropic salt which is an irritant. Always wear gloves while handling these reagents and avoid any skin contact!

Lysis Solution DC

warning
H302-312-332-412 EUH 032 P273

Wash Buffer R1

danger
H302-312-332-412 EUH032 P273

H302: Harmful if swallowed.
H312: Harmful in contact with skin.
H332: Harmful if inhaled.
H412: Harmful to aquatic life with long lasting effects.
EUH032: Contact with acids liberates very toxic gas.
P273: Avoid release to the environment.

Emergency medical information can be obtained 24 hours a day from infotrac:

outside of USA: **1 – 352 – 323 – 3500**
in USA : **1 – 800 – 535 – 5053**

Product characteristic of the InviTrap® RNA Tissue HTS 96 Kit/ C

Starting material	Yield	Time
1 mg - 10 mg fresh or frozen tissue sample	e.g. 30 µg total RNA from mouse liver tissue	about 50 min

The **InviTrap® RNA Tissue HTS 96 Kit/ C** is designed to isolate high yield of cellular total RNA from 1 mg up to 10 mg fresh or frozen tissue sample in a 96-well format.
No enzymatic step (DNase digestion) to remove contaminated genomic DNA is necessary.
Special buffer conditions guarantee an efficient lysis of the starting material and an inactivation of endogenous RNases. Genomic DNA contaminations are almost completely separated from the total RNA by binding to specially optimized mineralic carrier particles.

Downstream Application

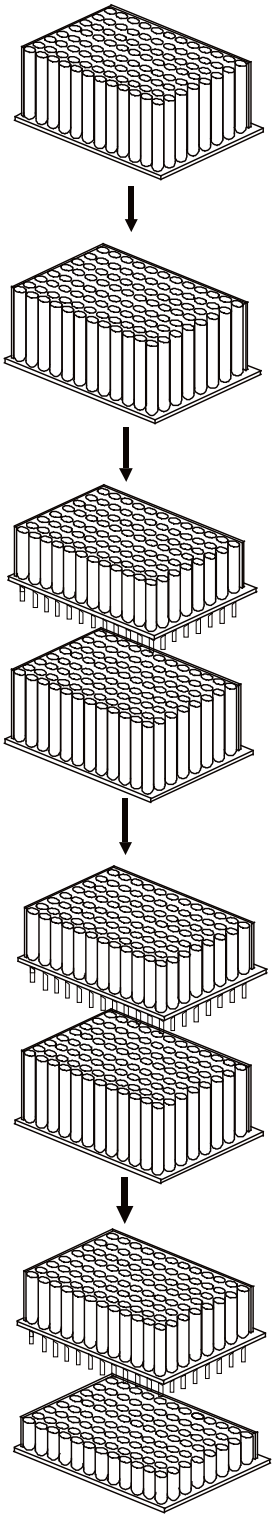
- RT-PCR
- DDRT-PCR
- Real-time PCR
- Northern Blot

Reagents and equipment to be supplied by user

- Multichannel pipet with tips
- Reagents reservoirs for multichannel pipets
- Centrifuge for microplates e.g. Eppendorf 5804/ 5804 R or 5810/ 5810 R centrifuge with deepwell-plate-rotor
- Ethanol (96-100%)
- dd H_2O
- Liquid N_2
- Mill for homogenization of starting material under liquid N_2

*) The PCR method is covered by U.S. Patents 4,683,195 and 4,683,202 owned by Hoffmann-LaRoche Inc. The purchase of the InviTrap® RNA Tissue HTS 96 Kit/ C cannot be construed as an authorization or implicit licence to practice PCR under any patents held by Hoffmann-LaRoche Inc.

Scheme of the InviTrap® RNA Tissue HTS 96 Kit/ C



1. Lysis
2. Adjust RNA binding conditions
3. Binding of total RNA to the RNA Binding Plate A
4. Wash steps to remove contaminants (1 x Wash Buffer R1, 1 x Wash Buffer R2)
5. Elution of total RNA

Please work quickly and perform all extraction steps at room temperature (RT).

Protocol : **RNA extraction from 1 mg-10 mg tissue material**

Important: *Shake Lysis Solution DCT gently before use!*
Tissue sample can be stored under Lysis Solution DCT at –20 °C for several month.

1. Tissue disruption
Grind sample under liquid nitrogen thoroughly to a fine powder using a mortar and pestle or using commercial available homogenizers (e.g. mills). Incomplete grinding of the tissue will lead to reduced total RNA yield! Transfer the tissue powder (max. 10 mg) into the cavities of the 2 ml Collection Plate.

2. Lysis of starting material

Important: *Before every use mix Lysis Solution DCT thoroughly by inverting the bottle 5-10 times!*

Add 0.5 ml Lysis Solution DCT to each cavity. Mix several times by pippetting up and down. Incubate for 15 min at RT. Centrifuge at maximum speed for 5 min.Transfer 450 µl from the supernatant into a **new** 2 ml Collection Plate very carefully. Avoid carryover of unlysed material.

3. Adjust RNA binding conditions
Add 450 µl Binding Buffer R to each well of the collection plate and mix the solution very well by pipetting several times using a multichannel pipet.
Place the RNA Binding Plate Aon the top of a 2 ml Collection Plate .
Transfer the samples completely into each well of the RNA Binding Plate A. Close the RNA Binding Plate A with the Plate Lid and incubate for 1 min at RT.
Load the whole block (RNA Binding Plate A/2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket.
Centrifuge at 4.000 rpm for 5 min at RT. Take the RNA Binding Plate A /2 ml Collection Plate out of the centrifuge. Remove the Plate Lid and discard the filtrate. Place the RNA Binding Plate Aback to the top of the 2 ml Collection Plate.

4. First washing of the RNA Binding Plate A
Add 500 µl Wash Buffer R1 to each well of the RNA Binding Plate C. Close the RNA Binding Plate Awith the Plate Lid. Load the whole block (RNA Binding Plate C/ 2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket.
Centrifuge at 4000 rpm for 5 min at RT. Take the RNA - Binding Plate out of the centrifuge, open the Plate Lid and place the plate on a clean paper towel.
Discard the filtrate and place the RNA Binding Plate Aback to the top of the 2 ml Collection Plate.

5. Second washing of the RNA Binding Plate C
Add 700 µl Wash Buffer R2 to each well of the RNA Binding Plate C. Close the RNA Binding Plate Awith the Plate Lid. Load the whole block (RNA Binding Plate A/ 2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket.
Centrifuge at 4.000 rpm for 5 min at RT. Take the RNA - Binding Plate out of the centrifuge, open the Plate Lid ad place the plate on a clean paper towel.

6. Removing of ethanol
Empty the 2 ml Collection Plate and dry its upper side with paper.
Place the RNA Binding Plate Aon top of the 2 ml Collection Plate and close it with the Plate Lid.
Load the whole block (RNA Binding Plate A/2 ml Collection Plate) into the holder and place the whole assembly in the rotor bucket of the centrifuge. Centrifuge at maximum speed for at least 15 min at RT to eliminate any traces of ethanol. Take the RNA Binding Plate A/2 ml Collection Plate out of the centrifuge, remove the Plate Lid and place the plate on a clean paper towel.
Discard the 2 ml Collection Plate.

7. Elution of the total RNA
Place the RNA Binding Plate Aon top of a RNase-free Elution Plate L.
Add 80 µl Elution Buffer R (RNase-free) **directly onto the membrane** in each well and incubate for 5 min. Close the RNA Binding Plate A with the Plate Lid and place the whole block (RNA Binding Plate A/ Elution Plate L) in the rotor bucket of the centrifuge. Centrifuge for 5 min at 4000 rpm.

Take the RNA Binding Plate A and the Elution Plate L out of the centrifuge very carefully in order to avoid cross-contaminations with adherent fluid. Discard the RNA Binding Plate Awith the Plate Lid. Take the Elution Plate L with the eluted total RNA and cover the plate with sealing foil or the cover. Store at –80°C.

Troubleshooting

Problem/probable cause	Comments and suggestions
Clogged Plate-Filter	
insufficient disruption or homogenisation	After lysis spin lysate to pellet debris and continue with the protocol using the supernatant. Reduce amount of starting material.
Little or no total RNA eluted	
insufficient disruption or homogenisation	Reduce amount of starting material. Overloading reduces yield!
incomplete elution	Prolong the incubation time with Elution Buffer R to 5-10 min or repeat elution step once again.
DNA-contamination	
to much material	Reduce amount of starting material. DNase digests the eluate containing the total RNA.
Total RNA degraded	
RNA source inappropriately handeled or stored	Ensure that the starting material is frozen immediately in liquid N_2 and is stored continuously at –80°C! Avoid thawing of the material. Ensure that the protocol, especially the first steps, has been performed quickly.
RNase contaminations of solutions, receiver tubes etc	Use sterile, RNase-free filter-tips. Before every preparation clean up the pipets, the devices and the working place. Always wear gloves!
Total RNA does not perform well in downstream-applications (e.g. RT-PCR)	
Ethanol carryover during elution	Increase g-force or centrifugation time in step 8.
Salt carryover during elution	Ensure that Wash Buffer R1 and R2 are at room temperature. Check up Wash Buffer R1 and R2 for salt precipitates. If there are any precipitates solve these precipitates by careful warming.

Appendix

General notes and safety recommendations on handling RNA

RNA is far less stable than DNA. It is very sensitive to degradation by endogenous RNases in the biological material and exogenous RNases which are permanently present everywhere in the lab. To achieve satisfactory qualitative and quantitative results in RNA preparations, contaminations with exogenous RNases have to be reduced as much as possible in accordance with the following recommendations:

- Always wear latex or vinyl gloves while handling reagents and RNA samples to prevent RNase contaminations from surface of the skin or from dusty laboratory equipment.
- Change gloves frequently and keep tubes closed.
- Keep isolated RNA on ice when aliquots are pipetted for downstream applications.
- Reduce preparation time as much as possible.
- Use only sterile, disposable polypropylene tubes throughout the procedure (these tubes are generally RNase free).
- Non-disposable plasticware should be treated before use to ensure that it is RNase-free. Plasticware should be thoroughly rinsed with 0.1 M NaOH, 1 mM EDTA followed by RNase-free water. You can also take chloroform-resistant plasticware rinsed with chloroform to inactivate RNases.
- All glassware should be treated before use to ensure that it is RNase-free. Glassware should be cleaned with detergent, thoroughly rinsed and oven baked at 240°C for four or more hours before use. Autoclaving alone will not completely inactivate many RNases. Oven baking will both inactivate RNases and ensure that no other nucleic acids (such as Plasmid DNA) are present on the surface of the glassware. You can also clean glassware with 0.1% DEPC (diethyl pyrocarbonate). The glassware has to stand 12 hours at 37°C and then autoclave or heat to 100°C for 15 min to remove residual DEPC.
- Electrophoresis tanks should be cleaned with detergent solution (e.g. 0.5% SDS), thoroughly rinsed with RNase-free water, and then rinsed with ethanol and allowed to dry.
- All buffers must be prepared from DEPC-treated RNase-free ddH_2O.
- Avoid handling bacterial cultures, cell cultures or other biological sources of RNases in the same lab where the RNA purification has to be carried out.
- Do not use equipment, glassware and plasticware employed for other applications which might introduce RNase contaminations in the RNA isolation.

Storage of RNA
Purified viral RNA can be stored at -80°C and is stable for several years at this condition.

Ordering information

Product	Package size	Catalogue No.
InviTrap® RNA Tissue HTS 96 Kit / C	2 x 96 preps	7062300200
InviTrap® RNA Tissue HTS 96 Kit / C	4 x 96 preps	7062300300
InviTrap® RNA Tissue HTS 96 Kit / C	24 x 96 preps	7062300400
InviMag® RNA Universal mini Kit/ KF96	1 x 96 preps	7460300100
InviMag® RNA Universal mini Kit/ KF96	5 x 96 prep	7460300200
InviTrap® Spin Universal RNA Mini Kit	10 preps	1060100900
InviTrap® Spin Universal RNA Mini Kit	50 preps	1060100200
InviTrap® Spin Universal RNA Mini Kit	250 preps	1060100300

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